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We have conceived of an approach to prepare novel compounds for the estrogen receptor that might be useful in the imaging and diagnosis of breast cancer, by the creation of steroidal-based imaging agents which take advantage of the desirable properties of gallium as a radioisotope.

We have completed the preparation of the carbon skeletons needed to assemble several of these compounds. Synthetic routes to ligands for model gallocycles have also been completed. Initial RBA data for several of these compounds have also been obtained.

Future efforts will be directed at completing the synthesis of the gallium-containing compounds of the D-ring class, which appear to be the most promising, and preparation of these compounds in radioactive form.

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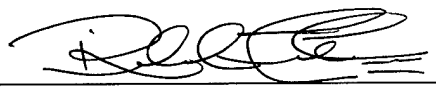

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TABLE OF CONTENTS

ANNUAL REPORT: October 1, 1998 – September 30, 1999

PRINCIPAL INVESTIGATOR: Richard R. Cesati III

TITLE: Gallium-Containing Estrogens as Receptor-Based Breast Tumor Imaging Agents

ORGANIZATION: University Of Illinois

INTRODUCTION, GOALS OF THE PROJECT AND APPROACH

[NOTE: For the purpose of continuity, the Table, Figure and first two Schemes in the attached Appendix have been taken directly from the original proposal. Schemes and figures in which new results are presented are also appended. Compound numbering refers only to those compounds shown in the new Schemes.]

Radioisotopes of gallium, as the trication Ga^{3+}, are regularly used for medical imaging, and other gallium compounds are being investigated for their potential as radiopharmaceuticals.[1-3] These radiodiagnostic agents consist of ligands chelated to either ^{67}Ga or ^{68}Ga radioisotopes. [4-6] Since the ^{66}Ga and ^{68}Ga isotopes are positron-emitting nuclides with convenient half-lives, we became interested in incorporating gallium into estrogens for imaging of breast tumors by PET.

Breast tumors can be imaged by the binding of appropriately labeled estrogens to the estrogen receptor (ER), which is found in many tumors, and these images provide useful prognostic information concerning cancer stage and tumor responsiveness to hormone therapy. [4,7-12] So far, however, breast tumor imaging through the ER has been accomplished only with a number of radiohalogen-labeled estrogens. The availability of the halogen radionuclides needed to produce these ER-binding agents, however, is limited, either by their short half-lives (e.g., for ^{18}F, $t_{1/2}$ = 110 min) or by difficulties in their production (e.g., production of ^{123}I requires high energy cyclotrons). Because ^{66}Ga and ^{67}Ga have relatively long half-lives ($t_{1/2}$ = 9.4 h and 3.3 days, respectively) and ^{68}Ga ($t_{1/2}$ = 68 min) is available from a long-lived ^{68}Ge ($t_{1/2}$ = 288 days) generator, replacing the radiohalogen in these imaging agents with these gallium radionuclides would make them more widely available and might also simplify their preparation.

When gallium is used as the citrate salt of the Ga^{3+} cation, most *in vivo* transport and uptake of this cation is mediated by iron-binding proteins, principally transferrin, because of the similar ionic radii of Ga^{3+} and Fe^{3+}.[13] If undesirable for a given procedure, binding of Ga^{3+} to transferrin

can be prevented by the use of high-affinity multidentate ligands, generally hexadentate ones. [14] An alternative form of gallium that has been evaluated for radiopharmaceutical purposes is $(CH_3)_2Ga^+$, a cation in which the metal-carbon bonds are highly stable toward hydrolysis.[1,15] The charge and size of this cation apparently make it sufficiently dissimilar to Fe^{3+} to prevent significant binding by plasma proteins.[15] Low affinity for plasma proteins, a tetracoordinate (rather than hexacoordinate) geometry, and the alkyl ligand environment of the metal suggest that dimethylgallium compounds may be more adaptable to the design of ligands for biological receptors than are multidentate Ga^{3+} complexes.

The highly stable metal-carbon bonds in dimethylgallium hints at further elaboration into actual steroidal and non-steroidal skeletons whereby a single gallium atom would cause minimal perturbation of the estrogenic framework of high affinity ligands. These chemical properties, in combination with the desirable properties of gallium as a radiolabel, should provide an effective means of imaging ER^+ tumors.

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BODY

Experimental Approach

Considerations in the Design of Gallium Compounds that Mimic ER Ligands

The estrogen template outlined in Figure 1 indicates that the gallium-containing estrogens should mimic the estradiol molecule or include a bis-phenolic structure. Within this framework, there are two methods of characterizing these estrogens: by carbon skeleton (steroidal or nonsteroidal), and by metal incorporation site (conjugated or integrated). Conjugate structures consist of a preformed active estrogen to which a metal fragment (moiety) is attached or "conjugated" usually by an alkyl spacer. In an "integrated" design, the metal is actually part of the molecular framework that gives the estrogen its affinity for ER.

In the original proposal we intended to systematically vary the combinations of the estradiol structure, using the integrated design, in conjunction with variations in the gallium moiety to produce the representative gallium-containing estrogens shown in Figure 1. Schemes 1 and 2 detail the well precedented synthetic routes to most of the target structures. The transformations involving gallium also have strong literature precedent, although novel gallium chemistry may need to be developed, especially at the tracer level.

The design of the estradiol mimics was based on the following criteria: (1) known high affinity for ER; (2) steric tolerance of substituents, (7α, 14α and 17α); and (3) ease of elaboration

into know gallium chemistry. The topological similarity of gallium and carbon, with respect to coordination geometry, is a key feature in "disguising" the radiolabel in this design: another desirable feature of the integrated design (Table 1).

The gallium moieties presented in Figure 1 were selected, as well, for their anticipated kinetic and/or thermodynamic stability on the basis of literature precedent as well as previous results from our laboratory. The use of bis-Grignard reagents in the preparation of six-membered gallocycles has extensive precedent, as does the use of intramolecular chelation as a means of stabilization of the gallium. In the original proposal, some reservations in the use of these bis-Grignard reagents, (possible side-reactions due to intermolecular cross-coupling, cyclization and polymerization), was presented, however, models studies to date indicate that these systems should behave as desired.

In vivo dissociation of a gallium moiety from the molecular frameworks shown in Figure 1 may still present a problem, despite the design efforts to maximize stability. Hydrolytic instability could be counteracted with structural modifications that stabilize gallium's coordination environment. Chelating N,N-dialkylamino groups are known to improve the stability of four-coordinate compounds toward hydrolysis,[16] and this fact could guide the modification or redesign of the targeted compounds. Another possible approach that is not developed in Figure 1 is to place gallium in a chelating amino acid moiety such as glycinatodimethylgallium.[17] In any case, extra tuning of a compound's properties could be achieved with electron-withdrawing groups or electron-donating groups that change the Lewis basicity of the ligand for gallium.

As we discuss below, we have made good progress in the syntheses of compounds with the steroidal design, as well as obtained a good lead into the non-steroidal class.

Results and Discussion for Year 2

The goals in Year 2 of our Statement of Work are listed below; each point will be discussed in the following sections.

Year 2

- Complete syntheses for the proposed Receptor-Based Imaging Agents (RBIA's) using non-radioactive ^{69}Ga
- Measure the specific binding of nonradioactive RBIA's to ER using the standard Receptor Binding Affinity (RBA) assay
- Begin preparation of radioactive RBIA's using ^{67}Ga or ^{68}Ga

Complete syntheses for the proposed RBIA's using non-radioactive ^{69}Ga

Because of the rapid progress in the preparation of ligands for the Class IA integrated steroids achieved in year one, we have decided to focus on the completion of their syntheses and to explore their preparation by radiochemical methods.

In order to obtain a ligand suitable for use in radiochemical synthesis, an exchange of the protecting group on the phenol had to be made. The aryl methyl ether used previously was originally found in the commercially available 6-methoxy-1-tetralone (Scheme 3) and was carried through the entire ligand synthesis. Since the corresponding free phenol was also commercially available, our initial goal was to put in place a new protecting group at this point and carry it to the penultimate product **4** as shown previously. Several factors had to be accounted for with the group choice. Among these are stability to the acidic, basic and reducing conditions of the synthesis as well as rapid removal following the radiochemical reaction. Based on these strict requirements, the benzyl protecting group was chosen.

Previous radiochemical work in our group showed that the benzyl group could in fact be removed rapidly via hydrogenolysis using a highly active form of colloidal palladium. With this in mind, 6-hydroxy-1-tetralone was protected using benzyl bromide and potassium carbonate in 74% yield. The benzyl protected tetralone was then subject to the Horner-Emmons reaction using diethyl cyanomethylphosphonate to afford the α,β–unsaturated nitrile in 76% yield.

The next step in the original sequence (Scheme 3), the reduction of the α,β–unsaturated nitrile to the allyl amine **1** proved to be problematic with the benzyl ether in place. Although we could achieve successful reduction of the nitrile to the desired product, the reaction was complicated by the removal of the benzyl group, likely due to the presence of the aluminum chloride. The free phenol also proved to be quite water soluble and difficult to purify by silica gel chromatography. Due to these complications, an alternative protecting group was sought.

The silicon-based protecting groups are well known for their ability to be adapted for resistance to either basic or acidic conditions, [18] as well as their facile fluoride-mediated removal. With this in mind we chose the *tert*-butyldiphenylsilyl (TBDPS) group as protection for the phenol.

The TBDPS group was put in place using standard conditions, (TBDPSCl and imidazole), to afford the protected tetralone in and excellent yield of 97%. The Horner-Emmons reaction also proceeded without incident, providing the desired α,β–unsaturated nitrile in 81% yield. The troublesome reduction reaction proved to be straightforward with the TBDPS group in place, affording the allyl amine in a modest 76% yield. The two-step vinylogous Pictet-Spengler, (Scheme 4), reaction then afforded the desired β-amino ester in an overall yield of 70%. Although

the reaction was successful, the product existed as a mixture of endo- and exo- olefin isomers, which were extraordinary difficult to separate.

Concomitantly with the above investigation, was a study aimed at the selective removal of the aryl methyl ether from one of the intermediates along the original synthetic pathway. Considering the results of the above investigation, we chose to attempt to selectively unmask the phenol at the diester **2** stage.

To accomplish this we had to employ conditions that would not remove the ethyl esters. Using conditions developed by Fujita and coworkers, [19] a solution of the N-methyl diester in ethanethiol was exposed to aluminum bromide at low temperature. The reaction produced only a trace of the desired material with the major product being due to a retro-Michael reaction of ethyl acetate to produce a highly conjugated system, which proved to be a fatal intermediate. Switching to a weaker Lewis acid, (aluminum chloride), did not improve the results. It should also be noted that both the boron- and silicon-based demethylation reagents were also unsuccessful.

Since the esters were apparently complicating the demethylation conditions, we sought to attempt the protecting group removal using the reduced material. The diol **3** did in fact, prove to be amenable to the demethylation conditions. The most successful conditions were those using boron tribromide. The triol however, proved again to be an extremely hydrophilic compound and could not be extracted completely following an aqueous workup. The crude product therefore had to be used in the reprotection step.

Initial attempts to reprotect the triol as a silyl ether, afforded monoprotection of the diol moiety exclusively. In fact, under no conditions could selective silylation of the phenol be achieved without activation of the diol. Eventually it was found that the crude triol could be selectively benzylated on the phenol using benzyl bromide and potassium carbonate in quantitative yield for the two steps. It should be noted that the benzyl-protected diol could be successfully transformed into the dichloride.

The final remaining issue regarding completion of the ligand synthesis for the Class IA integrated steroids is the reduction of the olefin to produce the steroid skeleton with the correct trans geometry at the octahydro-isoquinoline ring junction.

Since the olefin is tetrasubstituted, any attempts to hydrogenate the olefin directly would likely fail. If however, suitable conditions for the isomerization of the olefin under the reaction conditions could be found, the trisubstituted olefin would likely be reduced. Since we were well aware of the lability of the olefin with regard to acid-catalyzed isomerization, we first attempted to hydrogenate the olefin under mildly acidic conditions. Exposure of the N-methyl diester to one atmosphere of hydrogen in glacial acetic acid afforded no reduced product. In fact, there was also no isomerized product detected in the reaction mixture. Several attempts with other platinum and

palladium catalysts at one atmosphere of hydrogen were also unsuccessful. Interestingly, the use of trifluoroacetic acid and triethylsilane also provided no detectable reduced product.

We reasoned that the conditions used above were far too mild to affect reduction of the olefin and decided upon a harsher dissolving metal approach. Exposure of the diester above to a lithium/ammonia solution provided in one step both the reduction of the esters to the corresponding diol as well as the reduction of the olefin to a single isomer. Although the reaction proceeded in rather low yield, it was the first successful reduction of the olefin we had seen. The reaction was also complicated by a small amount of demethylation of the final product. Further studies with the pre-reduced diol provided a greater yield of the desired product. The geometry of the octahydro-isoquinoline ring junction is still unknown.

Measure the specific binding of nonradioactive RBIA's to ER using the standard RBA assay

Although the preparation of the final RBIA's has not been completed at this time, we have determined the RBA of several of our intermediates along our synthetic pathways. Of note are the two tetrahydropyran (THP) analogs shown in Figure 2. The RBA values for these compounds are indeed quite low, indicating that the substrate is a poor one for the estrogen receptor. This is not surprising in light of the fact that the geometry of the THP ring may indeed be in the opposite chair form, thereby orienting the oxygen atom distally from the binding contact found in the estrogen receptor. Further RBA analyses will be performed on the actual RBIA's once their synthesis is completed.

Begin preparation of radioactive RBIA's using ^{67}Ga or ^{68}Ga

Unfortunately we have not yet begun to prepare any of the RBIA's in radioactive form. We are currently focusing our time on the development of simple model systems with which the tracer-level chemistry can be worked out. The following is a description of the preparation of the ligands for these model systems and how their application will assist us in the radiochemical synthesis of the RBIA's.

As shown in Scheme 6, these model systems can be rapidly prepared from readily available starting materials and encompass both three- and four-coordinate gallium complexes. Much of the proposed organogallium chemistry involves novel reaction sequences previously unknown in the literature. The substrates are designed based upon the use of a bis-Grignard reagent to incorporate the gallium moiety.

The preparation of the substrates is relatively straightforward and involves transformations having excellent literature precedent. The preparation of the benzyl-bis-(3-chloropropyl)-amine has been previously described in the literature[20] and will not be further discussed. The preparation of the 1,5-dihalo-3,3-dimethyl-pentanes is achieved via a short synthetic sequence. The diester is initially prepared from the diacid using standard esterification conditions in nearly quantitative yield. Reduction of this ester using lithium aluminum hydride provides the diol also in good yield. Both the dibromo and dichloro compounds were prepared from the diol via tosylation/bromination or thionyl chloride treatment respectively.

We decided to investigate both the chloro and bromo compounds to determine the best methodology for the formation of the bis-Grignard. We reason that although the chloro compounds may be sluggish in the formation of the bis-Grignard, they will be less likely than the bromo compounds to form cyclic products under the reaction conditions. We also need to work out hydrolysis conditions by which we can selectively produce the cyclic dialkylgallium hydroxides in preference to the ring opened organogallium compound.

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KEY RESEARCH OUTCOMES

Progress in Relation to the Statement of Work

The complete three year Statement of Work, presented in the original proposal of July 1997, is shown below:

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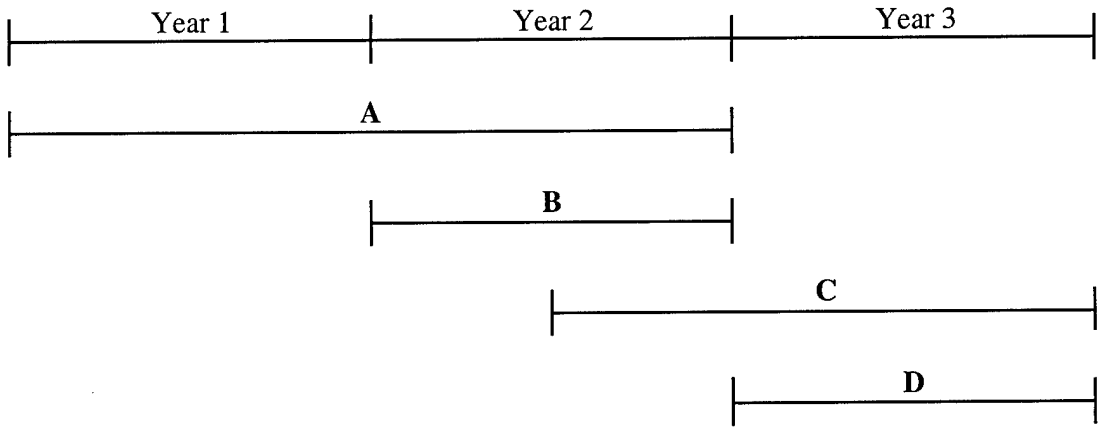
ORIGINAL STATEMENT OF WORK

Project Period: July 1, 1997– June 30, 2000 (3 years)



Prepare nonradioactive Ga compounds whose structures favor binding with the human estrogen receptor (ER).

- **Task A:** Months 1-24: Develop syntheses for the proposed Receptor-Based Imaging Agents (RBIA's) using non-radioactive ^{69}Ga.

Determine in vitro the estrogenicity of each Ga compound by measuring its Receptor Binding Affinity (RBA) and its non-specific binding.

- **Task B:** Months 13-24: The specific binding of nonradioactive RBIA's to ER will be measured by the standard Receptor Binding Affinity (RBA) assay.

Prepare radiolabeled samples of Ga-containing estrogens that have high RBAs and test their in vitro stability.

- **Task C:** Months 19-36: Synthesis of RBIA's will be achieved by repeating the most efficient techniques from Task A with ^{67}Ga or ^{68}Ga.

Evaluate radiolabeled Ga-containing estrogens as imaging agents for breast tumors.

- **Task D:** Months 25-36: Through our long-standing collaboration with Professor Michael Welch of the Mallinckrodt Institute of Radiology at Washington University Medical School we will evaluate the *in vivo* tissue distribution of RBIA's with promising *in vitro* properties.

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It is evident from the results presented in the preceding sections that progress has been slow in relation to that outlined in the Statement of Work for Year 2. We have worked out the final details for the ligand preparations for the Class IA integrated steroids; all of which should be amenable to the compounds in Class IIA. In addition, we have also obtained preliminary RBA data on our ligand classes. We have also prepared several model systems for the preparation of simple gallocycles as models for the tracer-level syntheses.

The key outcomes for the second year of this project

- Complete adaptation of initial ligand syntheses for radiochemical use.
- Preliminary RBA data on compounds from Class IA.
- Prepared model systems for tracer-level synthesis of novel gallocycles.

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CONCLUSIONS

From the results we have achieved so far, we have shown that the proposed synthetic routes are indeed viable methods for making the cores of these novel imaging agents. The synthetic routes outlined are now fully amenable to tracer-level synthesis. The RBA values for some of the members of these ligand classes has also been determined. Thus the project is progressing well along the lines initially envisioned in the original proposal.

REFERENCES

1. Cummins, C. H. Radiolabeled Steroidal Estrogens in Cancer Research. *Steroids* **1993**, *58*, 245 - 259.
2. Graham, M. C.; Pentlow, K. S. An investigation of the physical characteristics of ^{66}Ga as an isotope for PET imaging and quantification. *Med. Phys.* **1997**, *24*, 317-326.
3. Goethals, P.; Coene, M.; Slegers, G.; Vogelaers, D.; Everaert, J.; Lemahieu, I.; Colardyn, F.; Heyndrickx, G. R. Production of carrier-free ^{66}Ga and labeling of antimyosin antibody for positron imaging of acute myocardial infarction. *Eur. J. Nucl. Med.* **1990**, *16*, 237-240.
4. Helfrich, B.; Mitrowsky, A. Uber N-Glykoside. *Chem. Ber.* **1952**, *85*, 1 - 8.
5. Tobias, R. S.; Sprague, M. J.; Glass, G. E. Reactions of dimethylgallium(III) hydroxide. Raman, infrared, and proton magnetic resonance spectra of the dimethylgallium(II) aquo ion and several of its compounds. *Inorg. Chem.* **1968**, *7*, 1714 - 1721.
6. Tedesco, R.; Katzenellenbogen, J. A.; Napolitano, E. An expeditious route to 7α-substituted estradiol derivatives. *Tetrahedron Lett.* **1997**, *38*, 7997-8000.
7. Sun, Y.; Andersen, C. J.; Pajeau, T. S.; Reichert, D. E.; Hancock, R. D.; Motekaitis, R. J.; Martell, A. E.; Welch, M. J. Indium(III) and Gallium(III) Complexes of Bis(aminoethanthiol) Ligands with Different denticities: Stabilities, Molecular Modeling, and *inVivo* Behavior. *J. Med. Chem.* **1996**, *39*, 458-470.
8. Szelecsényi, F.; Boothe, T. E.; Tavano, E.; Plitnikas, M. E.; Tárkány, F. Compilation of cross sections/thick target yields for ^{66}Ga, ^{67}Ga and ^{68}Ga production using Zn targets up to 30 MeV proton energy. *Appl. Radiat. Isot.* **1994**, *45*, 473-500.
9. Haegle, E. Uber einige Condensationsproducte dir Amidophenole. *Chem. Ber.* **1892**, *25*, 2753 - 2758.
10. Harris, W. R.; Pecoraro, V. L. Thermodynamic Binding Constants for Gallium Transferrin. *Biochemistry* **1983**, *22*, 292 - 299.
11. Rijks, L. J. M.; Bakker, P. J. M.; Vantienhoven, G.; Noorduyn, L. A.; Boer, G. J.; Rietbroek, R. C.; Taat, C. W.; Janssen, A. G. M.; Veenhof, C. H. N.; Vanroyen, E. A. Imaging of estrogen receptors in primary and metastatic breast cancer patients with iodine-123-labeledZ-MIVE. *J. Clin. Oncol.* **1997**, *15*, 2536-2545.
12. Schumann, H.; Hartmann, U.; Wassermann, W.; Dietrich, A.; Gorlitz, F.; Pohl, L.; Hostalek, M. Intramolecularly Stabilized Organoaluminum, -gallium, and -indium Derivatives. *Chem. Ber.* **1990**, *123*, 2093 - 2099.
13. Green, M. A.; Welch, M. J. Gallium Radiopharaceutical Chemistry. *Nucl. Med. Biol.* **1989**, *16*, 435 - 448.
14. Rijks, L. J. M.; Sokole, E. B.; Stabin, M. G.; de Bruin, K.; Janssen, A. G. M.; van Royen, E. A. Biodistribution and dosimetry of iodine 123-labelled Z-MIVE: an oestrogen receptor radioligand for breast cancer imaging. *Eur. J. Nucl. Med.* **1998**, *25*, 40-47.
15. Green, M. A. *New Trends in Radiopharmaceutical Synthesis, Quality Assurance, and Regulatory Control*; Plenum Press: New York, 1991.
16. McKay, A. F.; Tarlton, E. J.; Petri, S. I.; Steyermark, P. R.; Mosley, M. A. Amino Acids. V. 1,3-Di-(w-carboxyalkyl)-thioureas and Their Chemistry. *J. Am. Chem. Soc.* **1958**, *80*, 1510-1517.
17. Rettig, S. J.; Storr, A.; Trotter, J. Synthesis and Structural characterization of Glycinatodimethylgallium, $(C_2H_4NO_2)Ga(CH_3)_2$. *Can. J. Chem.* **1986**, *65*, 1349-1352.
18. Kocie´nski, P. J. *Protecting Groups*; Thieme Verlag: Stuttgart, 1994.

19. Node, M.; Nishide, K.; Fuji, K.; Fujita, E. Hard Acid and Soft Nucleophile System. 2. Demethylation of Methyl Ethers of Alcohol and Phenol with Aluminum Halide-Thiol System. *J. Org. Chem.* **1980**, *45*, 4275-4277.
20. Groth, A. M.; Lindoy, L. F.; Meehan, G. V. New linked macrocyclic systmes derived from selectively protected S_2N_2 macrocycles. *J. Chem. Soc. Perkin Trans. 1* **1996**, 1553-1558.

APPENDICES

Table 1. Comparison of Ga Compounds with Requirements of Ga-Containing Estrogen – From the Original Proposal

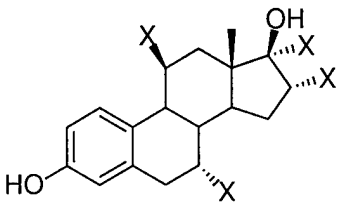
Property	Ideal Ga-Containing Estrogen	Ga Coordination Complexes	Ga Organometallics[1]
Ga Coordination Number	≤4	6	4
Stability in Aqueous Environment	high	high	high
Solubility at Physiological pH	high	variable[2]	high
Binding or Release of Ga to Transferrin	none	variable[2]	weak

1. Represented by the only such compound evaluated for all four properties: $(CH_3)_2Ga(C_5H_7O_2)$
2. May be low or high, depending on the presence of stabilizing ligands for Ga

Figure 1. Structural Diversity of Estrogens and Ga-Containing Analogs – From the Original Proposal

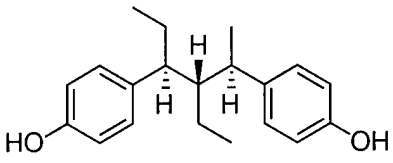
A. Estrogen

Steroidal



A. Estradiol

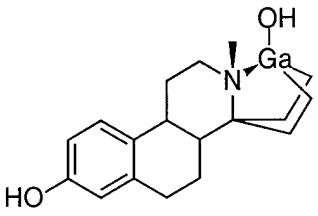
Three-Carbon Bridged



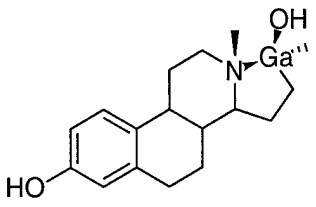
B. Benzestrol

B.Targeted Ga-Containing Analog

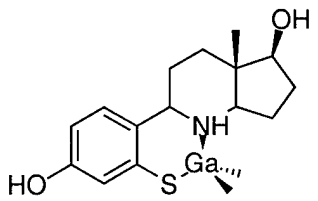
Steroidal Integrated



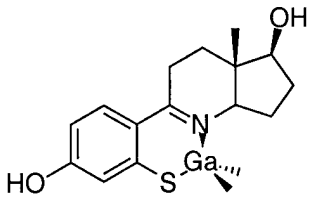
IA



IIA

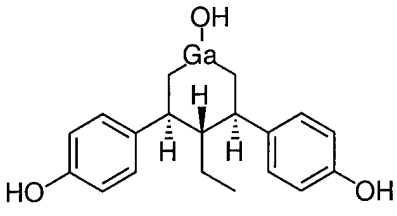


IIIA



IVA

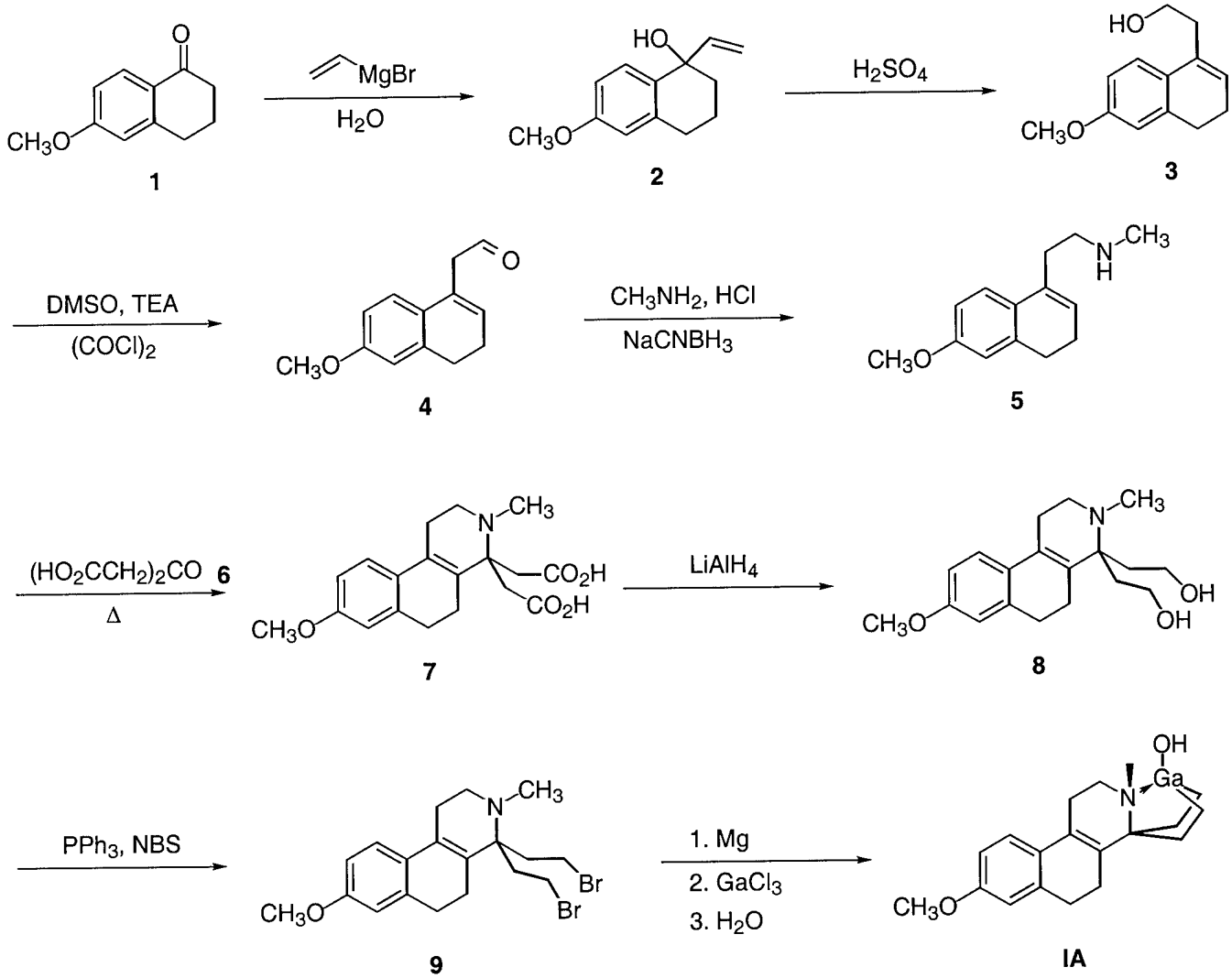
Nonsteroidal Conjugated



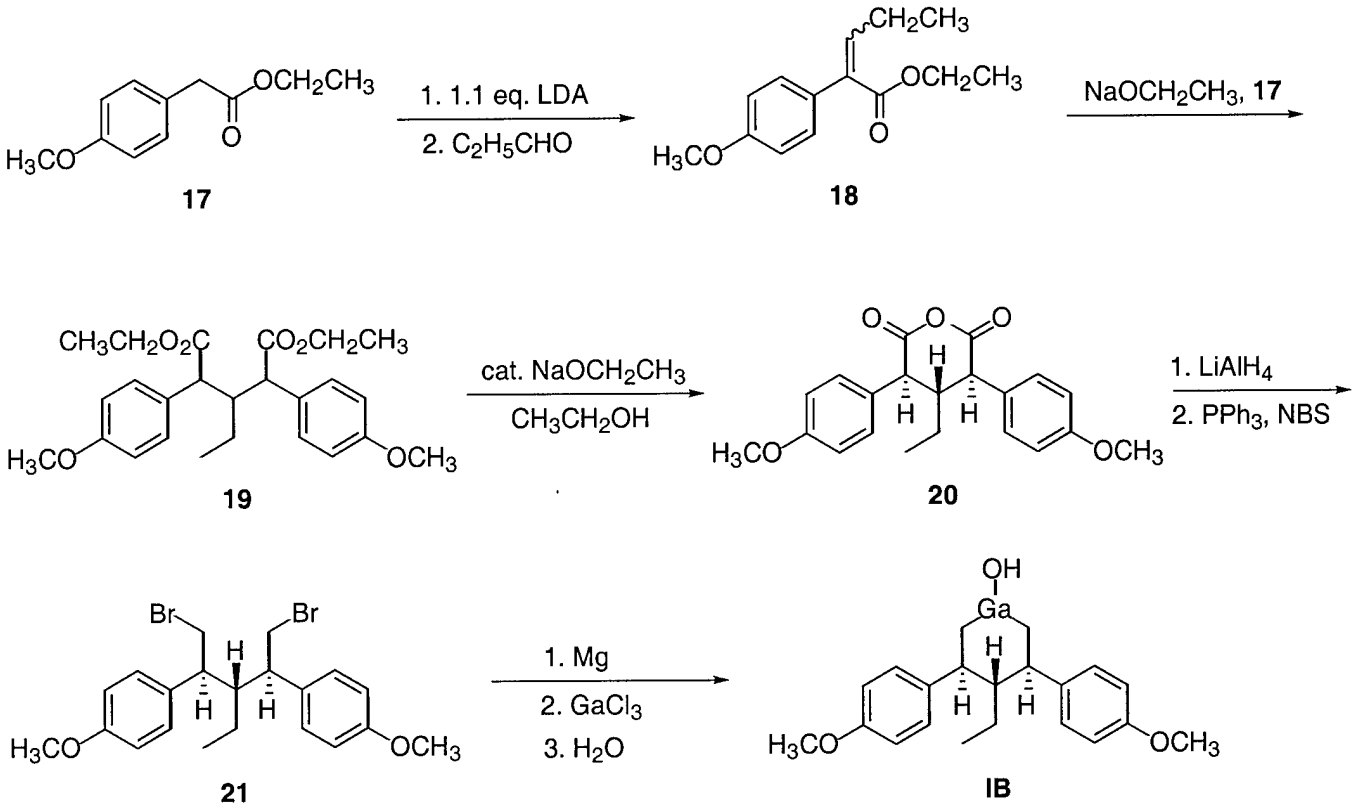
IB

X indicates a site where a large group may be placed without affecting affinity for ER

Scheme 1. Proposed Synthesis of Class IA – From the Original Proposal

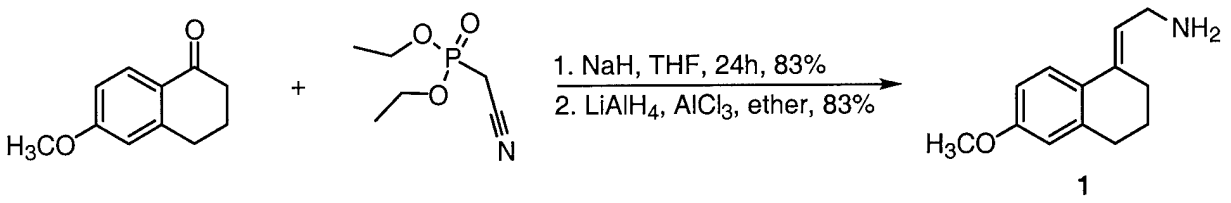


Scheme 2. Proposed Synthesis of Class IB – From the Original Proposal



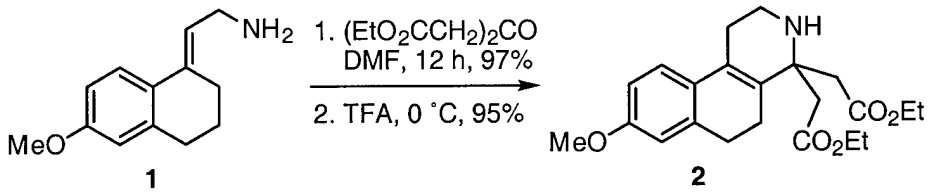
17 **18** **19** **20** **21** **IB**

Scheme 3. Preparation of Allyl Amine



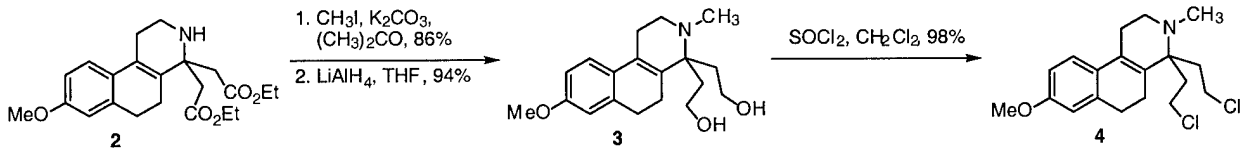
1

Scheme 4. Vinylogous Pictet-Spengler Reaction



1 2

Scheme 5. Preparation of Bis-Grignard Precursor



2 3 4

Scheme 6. Preparation of Bis-Grignard Models

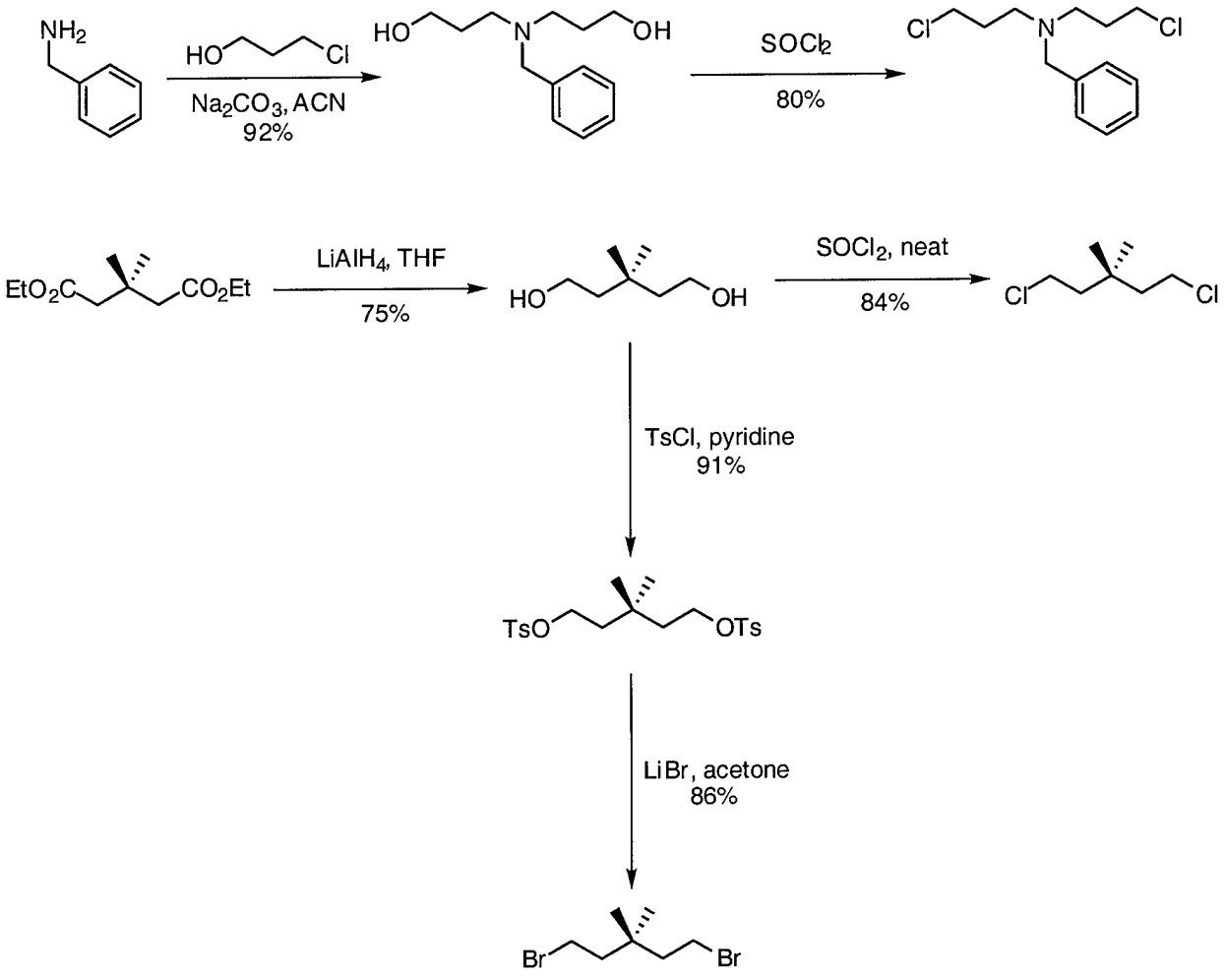
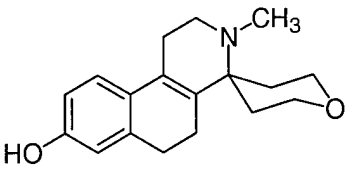
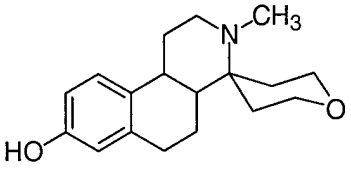


Figure 2. RBA Values of Intermediates

	RBA
	0.043
	~0.005